2. Bendix, P.M., Pedersen, M.S. & Stamou, D. Quantification of nano-scale intermembrane contact areas by using fluorescence resonance energy transfer. Proceedings of the National Academy of Sciences 106, 12341-12346 (2009).
3. Hatzakis, N.S. et al. How curved membranes recruit amphipathic helices and protein anchoring motifs. Nat. Chem. Biol. 5, 835-841 (2009).
4. Andreas H. Kunding, et al. Intermembrane docking reactions are regulated by membrane curvature. Biophysical Journal, in press.

1620-Pos Board B390

Calcium-Induced Calcium Release (CICR) Triggers Fusion of Individual Synaptic Vesicles in Rod Terminals

Minghui Chen[1], David Zenisek[2], Wallace B. Thoreson[1].
[1]Departments of Ophthalmology & Visual Sciences and Pharmacology & Experimental Neuroscience, University of Nebraska Medical Center, Omaha, NE, USA, [2]Department of Cellular and Molecular Physiology, Yale University, New Haven, CT, USA.

Photoreceptor light responses are encoded by changes in synaptic vesicle release. Release from rods is triggered by the opening of calcium channels beneath plate-like synaptic ribbons. Maintained depolarization can activate CICR and enhance release. Using total internal reflection fluorescence microscopy (TIRFM) to visualize release of single synaptic vesicles, we tested whether CICR enhances release from rods by stimulating fusion at non-ribbon sites.

Rods from salamander retina were loaded with activity-dependent dyes, FM1-43 or dextran-conjugated pHrodo, and visualized by TIRFM. Rods were depolarized with steps to −10 mV under voltage-clamp or by puff application of 50 mM KCl. CICR was activated with 10 μM ryanodine and inhibited with 100 μM ryanodine. Ribbon locations were identified with a fluorescent ribbon-binding peptide or from hot spots of depolarization-evoked calcium entry visualized with Fluo5F.

In terminals loaded with FM1-43 or pHrodo, depolarization stimulated rapid disappearance of vesicles with kinetics similar to that measured electrophysiologically. Additionally, stimulation-evoked vesicle disappearance was blocked by Cd^{2+}, indicating that it was due to calcium-dependent exocytosis. Vesicles docked for about 200 ms before fusion. Most release events occurred close to ribbons, but some also occurred further away. Activation of CICR with 10 μM ryanodine stimulated intracellular calcium increases and vesicle release. Ryanodine-evoked release events were less clustered than release evoked by depolarization, consistent with greater non-ribbon release. The spread of calcium evoked by 500 ms steps (but not 50 ms steps) was inhibited by blocking CICR with 100 μM ryanodine in the patch pipette. Release evoked by 500 ms steps also involved sites further from the ribbon than release evoked by 50 ms steps. These results indicate that the stimulation of CICR by maintained depolarization enhances release from rods by triggering fusion of vesicles at non-ribbon sites.

1621-Pos Board B391

The Role of Mobile Calcium Buffers in Synaptic Transmission at the Inner Hair Cell Ribbon Synapse

Tina Pangrsic[1], Nicola Strenzke[1], Beat Schwaller[2], Tobias Moser[1].
[1]University of Goettingen, Goettingen, Germany, [2]University of Fribourg, Goettingen, Switzerland.

Temporally precise sound encoding at the inner hair cell (IHC) ribbon synapse is tightly regulated by calcium. The mobile calcium buffers calbindin, parvalbumin alpha and calretinin might contribute to shaping the presynaptic Ca^{2+} signals. We investigated the function of these calcium binding proteins in IHC synaptic transmission by examining the auditory phenotype of double and triple buffer knockout mice. Our results show that buffer deficiency does not significantly alter hearing thresholds; however, we observed a slight increase in peak and steady-state sound-driven spike rates of spiral ganglion nerve fibers in knockout mice. The presynaptic function of IHCs was first studied by perforated patch-clamp recordings of Ca^{2+} currents and exocytic membrane capacitance increments. The absence of mobile calcium buffering proteins augmented sustained exocytosis in IHCs while leaving the amplitude and kinetics of exocytosis of the readily-releasable pool unchanged. Further, Ca^{2+}-dependent inactivation of calcium currents was stronger in IHCs of triple buffer knockout mice. In ruptured patch experiments we then tried to restore the calcium buffer capacity by adding exogeneous buffers. We estimated the concentration of endogenous buffers in IHCs to be equivalent to 0.5-1 mM BAPTA, which agrees well with previous estimates obtained by quantitative immunogold electron microscopy (Hackney et al., 2005). Our results demonstrate that calbindin, parvalbumin alpha and calretinin are involved in the regulation of synaptic transmission at the IHC ribbon synapse; however they do not seem to be essential for hearing, at least not during transient sound stimulation.

1622-Pos Board B392

Action Potential-Triggered Somatic Exocytosis in Mesencephalic Trigeminal Nucleus Neurons in Rat Brain Slices

Bo Zhang[1], Xiao-Yu Zhang[1], Wei Huang[1], Fei-Peng Zhu[1], Tao Liu[1], Yi-Ru Du[1], Bin Liu[1], Qi-Hui Wu[1], Xu-Yao Guo[1], Zu-Yin Chai[1], Hui Zheng[1], Pi-Fu Luo[2], **Zhuan Zhou**[1].
[1]Peking University, Beijing, China, [2]Department of Pathology, ACM Medical Laboratory, Rochester, NY, USA.

The neurons in the mesencephalic trigeminal nucleus (MeV) play essential roles in proprioceptive sensation of the face and oral cavity. The somata of MeV neurons are generally assumed to carry out neuronal functions but not to play a direct role in synaptic transmission. Using whole-cell recording and membrane capacitance (Cm) measurements, we found that the somata of MeV neurons underwent robust exocytosis (Cm jumps) upon depolarization and with the normal firing of action potentials in brain slices. Both removing $[Ca^{2+}]_o$ and buffering $[Ca^{2+}]_i$ with BAPTA blocked this exocytosis, indicating that it was completely Ca^{2+}-dependent. In addition, an electron microscopic study showed synaptic-like vesicles approximated to the plasma membrane in somata. There was a single Ca^{2+}-dependent releasable vesicle pool with a peak release rate of 1912 fF/s. Importantly, following depolarization-induced somatic exocytosis, GABA-mediated postsynaptic currents were transiently reduced by 31%, suggesting that the somatic vesicular release had a retrograde effect on afferent GABAergic transmission. These results provide strong evidence that the somata of MeV neurons undergo robust somatic secretion and may play a crucial role in bidirectional communication between somata and their synaptic inputs in the central nervous system.

1623-Pos Board B393

Calcein Inhibits Vesicle Release

Brooke A. Miller[1], Vytas P. Bindokas[2], Amy B. Harkins[1].
[1]Saint Louis University, St. Louis, MO, USA, [2]University of Chicago, Chicago, IL, USA.

Charged fluorescent dyes are frequently used as fluid phase makers to detect vesicle fusion events with the cell membrane by means of transient omega-like indentations. We used calcein (300 μM) to image vesicle fusion from nerve growth factor (NGF)-treated PC12 cells, a model secretory cell line. We stimulated the cells with either high K^+ saline or 100 μM nicotine to evoke calcium-dependent vesicle secretion. However, when the cells were stimulated in the presence of calcein, they showed a reduction in the number of vesicles that were released and/or endocytosed compared to cells that were stimulated in the absence of calcein. This observation from imaging experiments led us to hypothesize that calcein was inhibiting vesicle release from NGF-treated PC12 cells. In order to understand whether calcein was having an inhibitory affect on vesicle secretion and/or reuptake of vesicle membranes, we utilized amperometry to analyze vesicle release of catecholamine transmitter content from NGF-treated PC12 cells. Amperometry is an electrochemical detection method used routinely to measure release of vesicle contents from individual cells with a carbon fiber electrode. Analysis of amperometric spikes provides information as to the number of catecholamine molecules released and the kinetics of release. Our results show that calcein caused a reduction in the number of released amperometric spike events from 106 ± 13 events measured from control nicotine-stimulated cells, to 60 ± 11 events ($p<0.05$) in the presence of calcein. Peak amplitude, half-width, quantal content, or kinetics of rising or falling phase were unaffected by calcein. These data indicate that calcein has an inhibitory effect on vesicle fusion with the cellular membrane resulting in reduced amperometry events and a subsequent reduction in uptake of the fluid phase marker when used to track vesicles while imaging.

1624-Pos Board B394

Exocytic Mechanisms of Storage and Release of Brevetoxin in the Dinoflagellate Karenia Brevis

Kellie L. Vigna[1], Ivan Quesada[2], **Pedro Verdugo**[1].
[1]University of Washington, Friday Harbor, WA, USA, [2]Universidad Miguel Hernandez, Alicante, Spain.

Karenia brevis is a broadly distributed toxic dinoflagellate responsible for Red Tide outbreaks throughout the world. Deleterious effects of these blooms are caused by brevetoxin, a potent neurotoxin that binds to sodium channels in nerve and muscle cell leading to substantial marine life mortality and human

morbidity. Little is known about the mechanisms of storage and release of toxins in algal species responsible for harmful algal blooms. Toxins have been thought to be exported from these unicellulars by an ill-defined ''exudation'' mechanism. Although secretion is a standard strategy of material export in plants and animal cells, regulated exocytosis has only recently begun to be explored in dinoflagellates (FEBS Letters 2006, 580:2201-2206). Results presented here using fluorescently-labeled antibrevetoxin antibodies show that brevetoxin is present in *Karenia's* secretory vesicles and is released following blue light-stimulated exocytosis. The matrix of secretory granules functions as a caging polymer network that holds immobilized high payloads of active molecules including hormones, antibacterial peptides, or in this case toxins (Ann. Rev. Physiol 1990.52: 157-176). Upon release, *Karenia's* vesicle matrix undergoes typical phase transition from condensed to solvated phase, with characteristic first order kinetics swelling, and release of its brevetoxin payload. These observations support the notion that *Karenia b* functions as a typical secretory cell, opening the way for a better understanding of Red Tide blooms dynamics.

1625-Pos Board B395

Influenza Virus Hemagglutinin Delays Endosomal Acidification - a Strategy for Successful Delivery of the Viral Genome?

Christian Sieben[1], Thibault Lagache[2], David Holcman[2], Andreas Herrmann[1].
[1]Humboldt-University Berlin, Berlin, Germany, [2]Ecole Normale Supérieure, Paris, France.

Upon endocytic uptake of influenza virus, acidification of the endosomal lumen triggers a conformational change of the virus spike protein hemagglutinin (HA) leading to fusion between the endosomal and the viral membrane. For efficient infection, release of the viral genome favorably occurs in the vicinity of the nucleus to prevent lysosomal degradation of the viral RNA and activation of the cellular antiviral response. How influenza viruses ensure optimal duration of endosomal residence to avoid premature as well as delayed fusion and release of the genome is not understood.

The tight packing of HA in the viral envelope represents a remarkably high intra-endosomal protein concentration with high buffering potential for incoming protons. By using pH sensitive fluorescent markers we could show for the first time that the presence of a virus inside an endosome drastically slows down the acidification kinetics. We investigated the effect of cytoskeletal inhibitors on virus fusion and infection using a combination of single virus tracking and an intracellular fusion assay. In control cells, fusion mostly occurs in the perinuclear region. Inhibition of endosomal transport along microtubules by nocodazole did not change the numbers of fusion events, but randomized their localization within the cell. Interestingly, this dislocation correlates with strongly reduced infection efficiency, confirming that the site of virus-endosome fusion indeed plays an important role in the delivery of the viral genome.

Taken together, our results demonstrate that influenza virus HA delays the endosomal acidification to ensure timely as well as locally optimal release of its genome. This suggests a general function of the high-density packing of spike proteins that is characteristic of enveloped viruses infecting via the endocytic route.

1626-Pos Board B396

Role of Rac1-GTPase in Glucose Inhibition of Glucagon Secretion

Amicia D. Elliott, David W. Piston.
Vanderbilt University, Nasvhille, TN, USA.

The significance of glucagon in glucose homeostasis is becoming ever clearer, yet the mechanisms underlying its secretion from pancreatic α-cells remain poorly understood. Clinical studies show that drugs that suppress glucagon secretion can restore normoglycemia in diabetic patients, suggesting a possible alternative to insulin treatments. Two classes of models have been proposed to explain glucose inhibition of glucagon secretion (GIGS), but both share a requirement for Ca2+ triggering. In contrast, data from our lab show that changes in α-cell Ca2+ activity do not correlate with GIGS. This suggests that glucagon secretion is suppressed downstream of membrane depolarization, potentially at the level of exocytosis. The F-actin modifying Rac1-GTPase can regulate insulin secretion from β-cells by direct action on the exocytotic machinery. It has been shown that cAMP can regulate Rac1 activation in β-cells through PKA activation. Additionally, glucose metabolism activates the cAMP pathway independently of Ca2+ oscillations, though the complete role of metabolic activity in exocytosis is unknown.

Since the α-cell and β-cell are closely related, we hypothesize that GIGS depends on cAMP signaling that leads to deactivation of Rac1 and down-regulation of exocytosis. To identify the role of Rac1 in regulating GIGS, we have developed EGFP-tagged dominant negative and constitutively active Rac1 viral constructs that we use to infect murine islets with RFP-labeled α-cells. This allows us to study directly the effects of Rac1-GTP and Rac1-GDP on α-cell glucagon secretion, Ca2+ activity, and glucose metabolism to determine their relative roles in Rac1-mediated GIGS. Additionally, we are characterizing the glucose dose-response of Rac1 activation in α-cells. Finally, to determine the role of cAMP signaling we are using cAMP and PKA agonists at low and high glucose to identify their effects on Rac1 activation state and glucagon secretion.

1627-Pos Board B397

Rapid Cell Surface Kv2.1 Recycling Observed by Single Molecule Tracking

Aubrey V. Weigel, Kari Ecklund, Michael M. Tamkun, Diego Krapf.
Colorado State University, Fort Collins, CO, USA.

We study the insertion and retrieval of voltage-gated potassium channels, Kv2.1, at the single molecule level. Kv2.1 channels are labeled with quantum dots (QDs) at an extracellular domain. We observe QDs being internalized by the cell and new QD-tagged channels being inserted into the membrane. Because labeling occurs solely on the cell surface, only recycled channels that were previously in the plasma membrane can carry emerging QDs. Controls with both GFP and QD labels indicate that newly arriving QDs are indeed Kv2.1 channels. Channels that are in the plasma membrane from the beginning of the experiment can be either recycled or newly synthesized channels, as we cannot separate between these two in this measurement. The residence time distribution of channels that are on the cell surface from the beginning of our measurements has a median of 119 s, whereas for recycled channels the median is only 81 s, a 32% reduction (n = 334). In both instances it is surprising how short the residence time is on the cell surface of these channels. We propose that rapid channel turnover, via recycling pathways, helps the cell to maintain specialized regions in the membrane, which are entropically unfavorable. We investigate the role of actin in Kv2.1 trafficking using actin polymerization inhibitors. Upon the application of 5 μM cytochalasin D and 80 μM swinholide A, we observe that the residence times of both newly synthesized and recycled proteins are significantly reduced. In cells treated with actin inhibitors, channels are no longer sequestered into specific microdomains. Thus, channel recycling may function as an important factor in membrane compartmentalization and may be enhanced by stimuli that disrupt this organization.

1628-Pos Board B398

Fence Model for Dynamic Exchange and Retention of GLUT4 in Plasma Membrane Domains

Vladimir A. Lizunov[1], Karin Stenkula[2], Samuel Cushman[1], Joshua Zimmerberg[1].
[1]National Institutes of Health, Bethesda, MD, USA, [2]Lund University, Lund, Sweden.

Using a glucose transporter, all mammalian cells take up glucose from the extracellular space for energy and metabolism. In this study we followed the dynamics of the surface distribution of insulin-regulated glucose transporter −4 (GLUT4), introduced as a photo-switchable GLUT4-EOS probe to learn how GLUT4 molecules diffuse and interact with GLUT4 domains resident in the PM. Using Fluorescence Photo-Activation Localization Microscopy (FPALM), we observed dynamic confinement and release of GLUT4 from these domains, and measured lifetime of GLUT4 molecules trapped inside the domains. Although, the average time molecules spend within the domain was several orders of magnitude longer than time expected from free diffusion estimates, the molecules within the domain exhibited high mobility, and experienced multiple reflections from the boundaries of the domain.

We further provide evidence that exocytosis serves as main route of delivery and protein-specific retention of GLUT4 in plasma membrane domains. Fusion of GLUT4 vesicles with existing domains resulted in selective retention of GLUT4, but not other proteins co-transported in the same vesicles. Interestingly, insulin-stimulated fusion of GLUT4 vesicles that took place outside of the domains resulted in complete dispersal and free diffusion of GLUT4 into the plasma membrane. Importantly, we show that for endocytosis, GLUT4 molecules had to redistribute back to the domains, as freely-diffusing GLUT4